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In Vitro Activity of Plazomicin against Gram-Negative and Gram-Positive Isolates Collected from U.S. Hospitals and Comparative Activities of Aminoglycosides against Carbapenem-Resistant *Enterobacteriaceae* and Isolates Carrying Carbapenemase Genes

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ABSTRACT Plazomicin and comparator agents were tested by using the CLSI reference broth microdilution method against 4,825 clinical isolates collected during 2014 and 2015 in 70 U.S. hospitals as part of the ALERT (Antimicrobial Longitudinal Evaluation and Resistance Trends) program. Plazomicin (MIC_{50}/MIC_{90}, 0.5/2 μg/ml) inhibited 99.2% of 4,362 *Enterobacteriaceae* at ≤4 μg/ml. Amikacin, gentamicin, and tobramycin inhibited 98.9%, 90.3%, and 90.3% of these isolates, respectively, by applying CLSI breakpoints. The activities of plazomicin were similar among *Enterobacteriaceae* species, with MIC_{50} values ranging from 0.25 to 1 μg/ml, with the exception of *Proteus mirabilis* and indole-positive *Proteeae* that displayed MIC_{50} values of 2 μg/ml. For 97 carbapenem-resistant *Enterobacteriaceae* (CRE), which included 87 isolates carrying bla_{KPC}, plazomicin inhibited all but 1 isolate at ≤2 μg/ml (99.0% and 98.9%, respectively). Amikacin and gentamicin inhibited 64.9% and 56.7% of the CRE isolates at the respective CLSI breakpoints. Plazomicin inhibited 96.5 and 95.5% of the gentamicin-resistant isolates, 96.9 and 96.5% of the tobramycin-resistant isolates, and 64.3 and 90.0% of the amikacin-resistant isolates according to CLSI and EUCAST breakpoints, respectively. The activities of plazomicin against *Pseudomonas aeruginosa* (MIC_{50}/MIC_{90}, 4/16 μg/ml) and *Acinetobacter* species (MIC_{50}/MIC_{90}, 2/16 μg/ml) isolates were similar. Plazomicin was active against coagulase-negative staphylococci (MIC_{50}/MIC_{90}, 0.12/0.5 μg/ml) and *Staphylococcus aureus* (MIC_{50}/MIC_{90}, 0.5/0.5 μg/ml) but had limited activity against *Enterococcus* spp. (MIC_{50}/MIC_{90}, 16/64 μg/ml) and *Streptococcus pneumoniae* (MIC_{50}/MIC_{90}, 32/64 μg/ml). Plazomicin activity against the *Enterobacteriaceae* tested, including CRE and isolates carrying bla_{KPC} from U.S. hospitals, supports the development plan for plazomicin to treat serious infections caused by resistant *Enterobacteriaceae* in patients with limited treatment options.

KEYWORDS aminoglycosides, plazomicin, carbapenem-resistant *Enterobacteriaceae*

The worldwide emergence of multidrug-resistant (MDR) organisms, including carbapenem-resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, highlights the need for new therapeutic options to treat infections (1). In the early 2000s, the Infectious Diseases Society of America recognized the urgent need for surveillance initiatives and new therapeutic options for the group of organisms known as ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species), which includes Gram-negative organisms as well as troublesome Gram-positive species (2).

New therapeutic options for Gram-positive and -negative organisms were recently

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approved for clinical therapy in the United States and Europe (3); however, developing new agents with broad-spectrum activity is important given the significance of adequate empirical treatment for successful patient outcomes (4, 5).

Aminoglycosides are broad-spectrum agents that have been used for several decades to treat serious infections caused by nonfastidious Gram-negative bacteria, staphylococci, enterococci, and viridans group streptococci. Aminoglycosides are also used in combination with other agents displaying synergistic activity with this class, such as β-lactams, fluoroquinolones, polymyxins, and vancomycin (3). The most common mechanisms of resistance to aminoglycosides are aminoglycoside-modifying enzymes (AMEs) that are broadly disseminated among Gram-negative and Gram-positive species and carried by mobile genetic structures that also often harbor β-lactamases and other resistance genes (6, 7).

Plazomicin is a semisynthetic aminoglycoside derived from sisomicin and contains structural modifications that make this molecule stable in the presence of most AMEs (8, 9). Plazomicin has *in vitro* activity against nonfastidious Gram-negative pathogens and *Staphylococcus* spp., including methicillin-resistant *S. aureus* isolates (10, 11).

In this study, we evaluated the activities of plazomicin and comparator antimicrobial agents tested against 4,825 clinical isolates collected in U.S. hospitals during 2014 and 2015. *Enterobacteriaceae* isolates displaying elevated carbapenem MIC values were evaluated for carbapenemase genes, and a separate analysis focused on the activities of plazomicin and comparators against these subsets.

RESULTS

Activities of plazomicin and comparator agents. Plazomicin (MIC_{50} and MIC_{90}, 0.5 and 2 μg/ml, respectively) inhibited 99.2% of 4,362 *Enterobacteriaceae* isolates tested at ≤4 μg/ml (Table 1). For common *Enterobacteriaceae* species, plazomicin inhibited 99.9% of the 1,506 *K. pneumoniae* isolates (MIC_{50} and MIC_{90}, 0.25 and 0.5 μg/ml, respectively), 99.9% of the 1,346 *Escherichia coli* isolates (MIC_{50} and MIC_{90}, 0.5 and 1 μg/ml, respectively), and 99.4% of the 359 *Klebsiella oxytoca* isolates (MIC_{50} and MIC_{90}, 0.5 and 0.5 μg/ml, respectively) at ≤4 μg/ml (Table 1).

Plazomicin inhibited all 104 *Enterobacter cloacae* species complex isolates (here *E. cloacae*) (MIC_{50} and MIC_{90}, 0.5 and 0.5 μg/ml, respectively) at ≤2 μg/ml and all 120 *Enterobacter aerogenes* (MIC_{50} and MIC_{90}, 0.5 and 1 μg/ml, respectively), 159 *Citrobacter freundii* species complex (MIC_{50} and MIC_{90}, 0.5 and 1 μg/ml, respectively), 145 *Citrobacter koseri* (MIC_{50} and MIC_{90}, 0.25 and 0.5 μg/ml, respectively), and 107 *Serratia marcescens* (MIC_{50} and MIC_{90}, 1 and 2 μg/ml, respectively) isolates at ≤4 μg/ml (Table 1).

Overall, plazomicin inhibited 90.7%, 91.1%, 98.4%, and 99.1% of the *Morganella morganii*, *Providencia* species, *Proteus mirabilis*, and *Proteus vulgaris* group isolates at ≤4 μg/ml, respectively (Table 1). Plazomicin MIC_{50} values for *Morganella morganii*, *Proteus mirabilis*, and *Proteus vulgaris* (2, 2, and 2 μg/ml, respectively) were identical to the MIC_{50} values displayed by amikacin (2, 2, and 2 μg/ml, respectively), while gentamicin (0.5, 1, and 0.5 μg/ml, respectively) and tobramycin (0.5, 0.5, and 0.5 μg/ml, respectively) (data not shown) had 2- to 4-fold-lower MIC_{50} values. Plazomicin displayed MIC_{50} values (range, 0.25 to 1 μg/ml) lower than those observed for amikacin (2 to 1 μg/ml) for all remaining *Enterobacteriaceae* species and were similar to those observed for gentamicin (0.5 μg/ml for all species) and tobramycin (range, 0.25 to 2 μg/ml). The MIC_{50} values of all 4 aminoglycosides were very similar (range, 1 to 2 μg/ml) for *Providencia* species isolates (data not shown).

Amikacin (MIC_{50} and MIC_{90}, 1 and 4 μg/ml, respectively), gentamicin (MIC_{50} and MIC_{90}, 0.5 and 4 μg/ml, respectively), and tobramycin (MIC_{50} and MIC_{90}, 0.5 and 4 μg/ml, respectively) inhibited 98.9%, 90.3%, and 90.3%, respectively, of the 4,362 *Enterobacteriaceae* isolates tested by using the CLSI breakpoints (Table 2). Among other comparator agents, meropenem (MIC_{50} and MIC_{90}, 0.03 and 0.06 μg/ml, respectively; 97.9% susceptible) and tigecycline (MIC_{50} and MIC_{90}, 0.25 and 1 μg/ml, respectively; 99.0% susceptible according to U.S. Food and Drug Administration [FDA] breakpoints) were most active against the *Enterobacteriaceae* isolates tested (Table 2).

TABLE 1 Antimicrobial activity of plazomicin tested against 4,825 clinical isolates collected in 70 U.S. hospitals during 2014 to 2015

Organism or group (no. of isolates)[a]	No. (cumulative %) of isolates at MIC (μg/ml) of:													MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128		
Enterobacteriaceae (4,362)	25 (0.6)	308 (7.6)	971 (29.9)	1,764 (70.3)	822 (89.2)	317 (96.4)	121 (99.2)	13 (99.5)	11 (99.8)	2 (99.8)	3 (99.9)	1 (99.9)	4 (100.0)	0.5	2
Klebsiella pneumoniae (1,506)	18 (1.2)	246 (17.5)	621 (58.8)	580 (97.3)	34 (99.5)	4 (99.8)	1 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	1 (99.9)	1 (100.0)	0.25	0.5
Escherichia coli (1,346)	1 (0.1)	6 (0.5)	75 (6.1)	641 (53.7)	534 (93.4)	81 (99.4)	6 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	2 (100.0)	0.5	1
Klebsiella oxytoca (359)	2 (0.6)	17 (5.3)	92 (30.9)	214 (90.5)	27 (98.1)	4 (99.2)	1 (99.4)	0 (99.4)	0 (99.4)	0 (99.4)	1 (99.7)	0 (99.7)	1 (100.0)	0.5	0.5
Enterobacter cloacae species complex (104)	0 (0.0)	14 (13.5)	30 (42.3)	51 (91.3)	8 (99.0)	1 (100.0)								0.5	0.5
Enterobacter aerogenes (120)	2 (1.7)	6 (6.7)	41 (40.8)	53 (85.0)	16 (98.3)	1 (99.2)	1 (100.0)							0.5	1
Serratia marcescens (107)	0 (0.0)	1 (0.9)	5 (5.6)	37 (40.2)	52 (88.8)	9 (97.2)	3 (100.0)							1	2
Citrobacter freundii species complex (159)	0 (0.0)	5 (3.1)	42 (29.6)	91 (86.8)	18 (98.1)	2 (99.4)	1 (100.0)							0.5	1
Citrobacter koseri (145)	2 (1.4)	12 (9.7)	63 (53.1)	58 (93.1)	6 (97.2)	3 (99.3)	1 (100.0)							0.25	0.5
Morganella morganii (118)			0 (0.0)	7 (5.9)	31 (32.2)	38 (64.4)	31 (90.7)	6 (95.8)	2 (97.5)	2 (99.2)	1 (100.0)			2	4
Providencia spp. (158)	0 (0.0)	1 (0.6)	0 (0.6)	18 (12.0)	27 (29.1)	54 (63.3)	44 (91.1)	5 (94.3)	8 (99.4)	0 (99.4)	1 (100.0)			2	4
Proteus mirabilis (124)		0 (0.0)	2 (1.6)	5 (5.6)	23 (24.2)	72 (82.3)	20 (98.4)	2 (100.0)						2	4
Proteus vulgaris group (116)			0 (0.0)	9 (7.8)	46 (47.4)	48 (88.8)	12 (99.1)	0 (99.1)	1 (100.0)					2	4
Pseudomonas aeruginosa (103)	0 (0.0)	1 (1.0)	3 (3.9)	1 (4.9)	3 (7.8)	17 (24.3)	49 (71.8)	17 (88.3)	7 (95.1)	4 (99.0)	0 (99.0)	0 (99.0)	1 (100.0)	4	16
Acinetobacter spp. (95)	1 (1.1)	0 (1.1)	7 (8.4)	14 (23.2)	17 (41.1)	25 (67.4)	7 (74.7)	11 (86.3)	4 (90.5)	4 (94.7)	1 (95.8)	0 (95.8)	4 (100.0)	2	16
Coagulase-negative staphylococci (72)	17 (23.6)	21 (52.8)	26 (88.9)	8 (100.0)										0.12	0.5
Staphylococcus aureus (69)		0 (0.0)	14 (20.3)	51 (94.2)	2 (97.1)	2 (100.0)								0.5	0.5
MRSA (30)		0 (0.0)	9 (30.0)	18 (90.0)	2 (96.7)	1 (100.0)								0.5	0.5
Streptococcus pneumoniae (66)								0 (0.0)	4 (6.1)	32 (54.5)	30 (100.0)			32	64
Enterococcus spp. (58)				0 (0.0)	1 (1.7)	8 (15.5)	7 (27.6)	9 (43.1)	5 (51.7)	8 (65.5)	16 (93.1)	4 (100.0)		16	64

[a]MRSA, methicillin-resistant *S. aureus*.

TABLE 2 Activities of plazomicin and comparator agents tested against *Enterobacteriaceae* isolates

Organism group (no. of isolates tested) and antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	% S using CLSI breakpoints[a]	% S using EUCAST breakpoints[a]
Enterobacteriaceae (4,362)					
Plazomicin	0.5	2	≤0.06–>128		
Amikacin	1	4	≤0.25–>32	98.9	98.0
Gentamicin	0.5	4	≤0.06–>8	90.3	89.2
Tobramycin	0.5	4	≤0.12–>8	90.3	87.1
Ceftazidime	0.12	16	≤0.015–>32	87.9	85.4
Meropenem	0.03	0.06	≤0.015–>32	97.6	97.9
Piperacillin-tazobactam	2	16	≤0.5–>64	92.0	89.2
Colistin	≤0.5	>8	≤0.5–>8		84.0
Tigecycline	0.25	1	≤0.06–4	99.0[b]	95.7
Levofloxacin	≤0.12	>4	≤0.12–>4	82.9	79.5
CRE (97)					
Plazomicin	0.5	1	≤0.06–>128		
Amikacin	16	32	0.5–>32	64.9	46.4
Gentamicin	2	>8	0.12–>8	56.7	50.5
Tobramycin	>8	>8	0.12–>8	13.4	11.3
Ceftazidime	>32	>32	16–>32	0.0	0.0
Meropenem	16	>32	1–>32	2.1	7.2
Piperacillin-tazobactam	>64	>64	32–>64	0.0	0.0
Colistin	≤0.5	>8	≤0.5–>8		78.1
Tigecycline	0.5	1	0.12–2	100.0[b]	94.8
Levofloxacin	>4	>4	≤0.12–>4	18.6	11.3
Isolates carrying bla_{KPC} genes (87)					
Plazomicin	0.25	1	≤0.06–>128		
Amikacin	16	32	0.5–>32	63.2	42.5
Gentamicin	4	>8	0.12–>8	55.2	48.3
Tobramycin	>8	>8	0.12–>8	11.5	9.2
Ceftazidime	>32	>32	4–>32	1.1	0.0
Meropenem	16	>32	0.5–>32	3.4	10.3
Piperacillin-tazobactam	>64	>64	64–>64	0.0	0.0
Colistin	≤0.5	>8	≤0.5–>8		78.2
Tigecycline	0.5	1	0.12–2	100.0[b]	96.6
Levofloxacin	>4	>4	≤0.12–>4	14.9	9.2
Carbapenemase-negative isolates (26)					
Plazomicin	0.5	1	0.12–2		
Amikacin	4	16	0.5–32	92.3	84.6
Gentamicin	1	>8	0.25–>8	69.2	69.2
Tobramycin	2	>8	0.25–>8	57.7	50.0
Ceftazidime	>32	>32	0.25–>32	19.2	15.4
Meropenem	2	32	0.03–32	23.1	53.8
Piperacillin-tazobactam	>64	>64	1–>64	19.2	15.4
Colistin	≤0.5	>8	≤0.5–>8		75.0
Tigecycline	0.5	2	0.06–2	100.0	88.5
Levofloxacin	2	>4	≤0.12–>4	50.0	34.6

[a]Criteria reported by the CLSI (14) and EUCAST (15). S, susceptible.
[b]Breakpoints from the FDA website (16).

Plazomicin had activity against the majority of *Enterobacteriaceae* isolates resistant to gentamicin and tobramycin (Table 3), inhibiting 96.5% and 95.5% of the gentamicin-resistant isolates according to CLSI and EUCAST breakpoint criteria, respectively, and 96.9% and 96.5% of the tobramycin-resistant isolates according to the same criteria. Only 14 isolates were resistant to amikacin when applying the CLSI criteria, and plazomicin inhibited 9 (64.3%) of these isolates at ≤4 μg/ml. Plazomicin inhibited 90.0% of the 50 amikacin-resistant isolates identified by using the EUCAST breakpoints at ≤4 μg/ml. Five amikacin-resistant isolates had plazomicin MIC values of ≥128 μg/ml and were resistant to gentamicin and tobramycin.

For 103 *P. aeruginosa* isolates, the plazomicin MIC_{50} and MIC_{90} values were 4 and 16 μg/ml, respectively. These values were 2-fold higher than those for amikacin and gentamicin (MIC_{50} and MIC_{90}, 2 and 8 μg/ml, respectively, for both) and 8- to 16-fold

TABLE 3 Activities of plazomicin and comparator aminoglycosides against phenotypes and genotypes of *Enterobacteriaceae* isolates and other Gram-negative species

Organism phenotype or genotype	Source of breakpoint criteria for aminoglycoside resistance (no. of isolates)[a]	% of isolates inhibited by plazomicin at ≤4 μg/ml	MIC_{50}/MIC_{90} (μg/ml)			
			Plazomicin	Amikacin	Gentamicin	Tobramycin
Enterobacteriaceae	NA (4,362)	99.2	0.5/2	1/4	0.5/4	0.5/4
Amikacin resistant	CLSI (14)	64.3	1/>128	>32/>32	>8/>8	>8/>8
	EUCAST (50)	90.0	0.5/4	32/>32	2/>8	>8/>8
Gentamicin resistant	CLSI (377)	96.6	0.5/2	2/8	>8/>8	>8/>8
	EUCAST (424)	95.5	0.5/2	2/8	>8/>8	>8/>8
Tobramycin resistant	CLSI (292)	96.9	0.5/2	4/32	>8/>8	>8/>8
	EUCAST (423)	96.5	0.5/2	4/32	>8/>8	>8/>8
CRE	NA (97)	99.0	0.5/1	16/32	2/>8	>8/>8
CPE (all carrying bla_{KPC})	NA (87)	98.9	0.25/1	16/32	4/>8	>8/>8
Carbapenemase negative	NA (26)	100.0	0.5/1	4/16	1/>8	2/>8
Pseudomonas aeruginosa	NA (103)	71.8	4/16	2/8	2/8	0.5/1
Acinetobacter baumannii	NA (99)	65.2	2/32	4/>32	1/>8	1/>8

[a]NA, not applicable.

higher than those for tobramycin (MIC_{50} and MIC_{90}, 0.5 and 1 μg/ml, respectively) (Tables 1 and 3). Amikacin (97.1% susceptible according to CLSI breakpoints), tobramycin (94.2% susceptible according to CLSI breakpoints), and colistin (98.1% susceptible according to EUCAST breakpoints) (data not shown) were the most active comparators against *P. aeruginosa* isolates.

For 95 *Acinetobacter* species isolates, plazomicin MIC_{50} and MIC_{90} values were 4 and 16 μg/ml, respectively (Table 1). Amikacin (MIC_{50} and MIC_{90}, 4 and >32 μg/ml, respectively), gentamicin (MIC_{50} and MIC_{90}, 1 and >8 μg/ml, respectively), and tobramycin (MIC_{50} and MIC_{90}, 1 and >8 μg/ml, respectively) (Table 3) inhibited 83.0%, 69.5%, and 77.9% of the isolates, respectively, according to CLSI breakpoint criteria. Among other comparators, only colistin (94.7% susceptible using CLSI or EUCAST criteria), ampicillin-sulbactam (72.3% using CLSI criteria), and imipenem (70.5% using both criteria) inhibited >70.0% of the isolates (data not shown).

All 72 coagulase-negative staphylococci (plazomicin MIC_{50} and MIC_{90}, 0.12 and 0.5 μg/ml, respectively) were inhibited by plazomicin at ≤0.5 μg/ml, and 69 *S. aureus* (plazomicin MIC_{50} and MIC_{90}, 0.5 and 0.5 μg/ml, respectively) (Table 1) isolates tested were inhibited by plazomicin at ≤2 μg/ml, including methicillin-resistant isolates (30 isolates tested) (Table 1). Gentamicin inhibited 72.2% and 97.1% of the respective coagulase-negative staphylococcus and *S. aureus* isolates tested at the CLSI breakpoints (data no shown).

Plazomicin had limited antimicrobial activity against *Streptococcus pneumoniae* (n = 66; MIC_{50} and MIC_{90}, 32 and 64 μg/ml, respectively) and *Enterococcus* spp. (n = 58; MIC_{50} and MIC_{90}, 16 and 64 μg/ml, respectively) (Table 1). As expected, other aminoglycosides also had activities similar to those of plazomicin against these organisms (data not shown).

Activity of plazomicin against carbapenem-resistant and carbapenemase-producing *Enterobacteriaceae*. A total of 97 (2.2% of *Enterobacteriaceae*) CRE isolates were identified in this study. Plazomicin (MIC_{50} and MIC_{90}, 0.5 and 1 μg/ml, respectively) inhibited 99.0% of these isolates at an MIC of ≤2 or ≤4 μg/ml (Table 2). CRE isolates were highly resistant to comparator agents, but nearly all of them were susceptible to tigecycline (100.0% susceptible according to U.S. FDA breakpoints) (Table 2). Amikacin (MIC_{50} and MIC_{90}, 16 and 32 μg/ml, respectively), gentamicin (MIC_{50} and MIC_{90}, 2 and >8 μg/ml, respectively), and tobramycin (MIC_{50} and MIC_{90}, >8 and >8 μg/ml, respectively) inhibited only 64.9%, 56.7%, and 13.4% of the isolates, respectively, at current CLSI breakpoints (Table 2).

Among 113 isolates displaying imipenem and/or meropenem MIC values of ≥2 μg/ml, carbapenemases were detected in 87 isolates, including 79 *K. pneumoniae*, 4 *K.*

oxytoca, 2 *C. freundii*, 1 *E. cloacae*, and 1 *S. marcescens* isolates. All isolates carried a bla_{KPC} variant: 56 carried bla_{KPC-3}, 29 carried bla_{KPC-2}, 1 carried bla_{KPC-4}, and 1 carried bla_{KPC-17}. These isolates were detected in all U.S. census divisions but were observed mainly in the Middle Atlantic division (52 isolates; 59.8% of the carbapenemase-producing *Enterobacteriaceae* [CPE]).

Plazomicin (MIC_{50} and MIC_{90}, 0.25 and 1 μg/ml, respectively) inhibited 86 of the 87 (98.9%) isolates carrying bla_{KPC} at $\leq$2 or $\leq$4 μg/ml (Tables 2 and 3). One isolate exhibited a plazomicin MIC at $>$128 μg/ml, was resistant to all aminoglycosides, and carried the 16S rRNA methyltransferase gene *rmtF1*. Amikacin (MIC_{50} and MIC_{90}, 16 and 32 μg/ml, respectively), gentamicin (MIC_{50} and MIC_{90}, 4 and $>$8 μg/ml, respectively), and tobramycin (MIC_{50} and MIC_{90}, $>$8 and $>$8 μg/ml, respectively) inhibited 63.2 and 42.5%, 55.2 and 48.3%, and 11.5 and 9.2% of the respective CPE isolates according to current CLSI/EUCAST breakpoint criteria.

Carbapenemase-negative isolates included 16 *K. pneumoniae* isolates, 4 *S. marcescens* isolates, and 3 isolates of other species. Plazomicin demonstrated comparable activities against the carbapenemase-negative (MIC_{50} and MIC_{90}, 0.5 and 1 μg/ml, respectively) and CPE (MIC_{50} and MIC_{90}, 0.25 and 1 μg/ml, respectively) isolates, and the highest plazomicin MIC value observed was at 2 μg/ml. In contrast, the aminoglycoside comparators were more potent against the 26 carbapenemase-negative isolates than against the CPE isolates. Amikacin (MIC_{50} and MIC_{90}, 4 and 16 μg/ml, respectively), gentamicin (MIC_{50} and MIC_{90}, 1 and $>$8 μg/ml, respectively), and tobramycin (MIC_{50} and MIC_{90}, 2 and $>$8 μg/ml, respectively) inhibited 92.3%, 69.2%, and 57.7% of the carbapenemase-negative isolates, respectively, when applying the CLSI breakpoints (Table 2).

DISCUSSION

Patients with prolonged hospitalization, including those in intensive care or long-term-care facilities, immunocompromised patients, and others with malignant conditions, often develop infections, and many of the infections are caused by MDR organisms (1). These organisms include members of the *Enterobacteriaceae* family, including CRE, and pandrug- and extensively drug-resistant *P. aeruginosa*, *A. baumannii*, and Gram-positive species, including *E. faecium* and *S. aureus*. The urgent need for monitoring initiatives and new potential therapeutic options for these organisms has been recognized by the medical and scientific communities, and although various new antimicrobial agents for Gram-positive infections have been approved, the number of non-β-lactam candidates for treating Gram-negative infections in late-stage development is still limited (2–5).

In this study, plazomicin displayed activity against CRE and CPE isolates detected in U.S. hospitals. The majority of the CRE isolates (and all CPE isolates) carried bla_{KPC}, and only 1 of these isolates had a plazomicin MIC value of $>$4 μg/ml. CRE isolates displayed elevated rates of resistance to all β-lactams and comparator agents, including gentamicin and amikacin, which inhibited 63.2% or fewer of the bla_{KPC}-carrying isolates at current CLSI or EUCAST breakpoints. Furthermore, plazomicin displayed activity against most isolates resistant to gentamicin and tobramycin according to CLSI or EUCAST breakpoints and inhibited $>$63% of the amikacin-resistant isolates.

Combination therapy is often used to treat infections caused by CRE, and this might include an aminoglycoside. The *in vitro* results presented here suggest that there is a potential role for this new agent in the antimicrobial armamentarium against difficult-to-treat MDR organisms. These data are supported by the results of 2 recent phase 3 clinical trials that evaluated plazomicin in complicated urinary tract infections and in serious infections due to CRE (i.e., bloodstream infections, hospital-acquired and ventilator-associated bacterial pneumonia, and complicated urinary tract infections) (ClinicalTrials.gov registration numbers NCT02486627 [https://clinicaltrials.gov/ct2/show/NCT02486627] and NCT01970371 [https://clinicaltrials.gov/ct2/show/NCT01970371]).

Plazomicin demonstrated activity against *Enterobacteriaceae*, *Staphylococcus* spp. regardless of methicillin resistance, and some *P. aeruginosa* and *Acinetobacter* species isolates. A tentative breakpoint of 4 μg/ml was applied during the plazomicin development program and thus was also applied here for the analysis of the *Enterobacteriaceae* population, based on pharmacokinetic/pharmacodynamic (PK/PD) data, which was supported by animal efficacy data (12). By applying this tentative breakpoint, the *in vitro* activity of plazomicin was similar to or greater than those of other aminoglycosides against important organism groups, which include multidrug-resistant isolates, that can pose a challenge for current antimicrobial chemotherapy options.

MATERIALS AND METHODS

Bacterial isolates. A total of 4,825 clinical isolates, including 4,362 *Enterobacteriaceae*, 265 Gram-positive cocci, 103 *P. aeruginosa* isolates, and 95 *Acinetobacter* spp., collected during 2014 and 2015 in 70 hospitals located in 61 U.S. cities were evaluated as part of the ALERT (Antimicrobial Longitudinal Evaluation and Resistance Trends) program. This surveillance program collects key pathogens in targeted numbers (1 per patient episode) deemed to cause urinary tract infections (1,414 isolates), bloodstream infections (1,178), pneumonia in hospitalized patients (1,125), skin and skin structure infections (566), and intra-abdominal infections (441). Other infection sources (101 isolates) were also accepted for uncommon species. Species identification was confirmed, when necessary, by matrix-assisted laser desorption ionization–time of flight mass spectrometry using the Bruker Daltonics MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA), according to the manufacturer's instructions.

Antimicrobial susceptibility testing. All isolates were tested for susceptibility to plazomicin and comparator agents using the reference broth microdilution method described by the CLSI (13). Categorical interpretations for all comparator agents were found in CLSI criteria in document M100 (14), EUCAST breakpoint tables (15), and/or the FDA website (16). Quality control was performed by using *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619. All quality control MIC results were within acceptable ranges as reported in CLSI documents.

Definitions. CRE was defined as any isolate exhibiting an imipenem, meropenem, or doripenem MIC value of >2 μg/ml. *Proteus mirabilis* and indole-positive *Proteeae* were selected by using meropenem and doripenem only due to the intrinsically elevated imipenem MIC values.

Characterization of carbapenemases. Isolates nonsusceptible to imipenem or meropenem were screened by PCR followed by DNA sequencing of bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-48}, bla_{GES} (bla_{GES-2}, bla_{GES-4}, bla_{GES-5}, bla_{GES-6}, and bla_{GES-8}), bla_{NMC-A}, bla_{SME}, and bla_{IMI} (17). Isolates yielding negative results for these genes were tested for less common carbapenemases, including genes encoding FRI-1, BKC-1, GIM-1/-2, SIM-1, SPM-1, KHM-1, AIM-1, BIC-1, and DIM-1 (18).

Amplicons generated were sequenced on both strands, and the nucleotide sequences obtained were analyzed by using the Lasergene software package (DNAStar, Madison, WI, USA) and compared to available sequences via an NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/).

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